IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants(s)	:	Lamb *et al.*
U.S. Serial No.	:	09/310,685
Filing Date	:	May 4, 1999
Examiner	:	F. Pierre VanderVegt
Art Unit	:	1644
For	:	**NOTCH**

745 Fifth Avenue
New York, NY 10151

EXPRESS MAIL

Mailing Label Number: EV 385418045 US

Date of Deposit: March 24, 2004

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Saddam Ahmed
(Typed or printed name of person mailing paper or fee)

S. Ahmed
(Signature of person mailing paper or fee)

DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Brian R Champion, declare and state that:

1. A copy of my *curriculum vitae* demonstrating my education, training and experience is of record in this application. I am familiar with U.S. application Serial No. 09/310,685, and its prosecution history. I am considered by my peers to be an expert in the field to which the application pertains, and am otherwise qualified to speak and render expert opinions as to the present application, invention, and issues of the Office Action dated September 24, 2003. Thus, this Declaration is in response to the Office Action.

2. The following experiments were performed by me or under my direction, supervision or control, and in the ordinary course of business.

(a) Dextran Coupling

A Delta1 protein fragment comprising the DSL domain and the first three EGF-like repeats (D1E3C) was coupled to dextran as follows. Derivatized maleimido-dextran was added to concentrated, reduced D1E3C at a 1:75 molar ratio of dextran to D1E3C. Coupling proceeded for 18h, 4 °C. The resulting D1E3C-dextran polymer (D1E3C-dextran conjugate; comprising aminodextrans each coupled to D1E3C proteins via SMCC linkers) was purified by gel permeation chromatography and screened for endotoxin contamination prior to activity assays *in vivo* as described below.

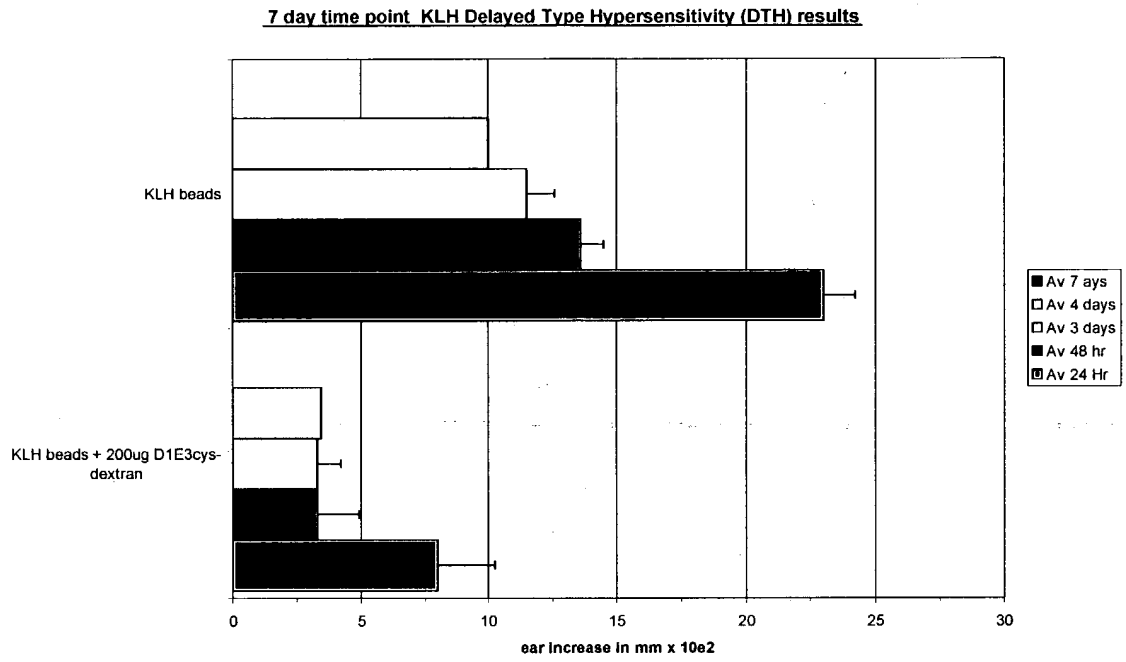
i) Coating of beads with KLH

Surfactant-free White Aldehyde/Sulfate Latex Beads were washed in PBS, resuspended in Imject® Mariculture Keyhole Limpet Hemocyanin (mcKLH), washed again and then resuspended at the required concentration. Successful coating of the beads was checked by their ability to neutralize an anti-KLH antiserum in an ELISA system.

ii) *In vivo* administration with D1E3C/dextran conjugate

6-8 weeks old female Balb/c mice were injected sub-cutaneously at the base of the tail with 2 x 10^6 KLH coated beads (prepared as described in (i) above) per mouse. Dextran-D1E3C conjugate prepared as above (approximately 200 μg per mouse), was injected sub-cutaneously in a close separate site of the tail base (all agents were administered as aqueous solutions;100 mM sodium phosphate at pH7).

Mice were challenged after 7 days in the right ear with 20 μg of KLH. The increase in ear swelling (right ear – left ear) was measured for the following four days using a digital calliper. Results are shown below (mice which received KLH beads alone are shown as a control):



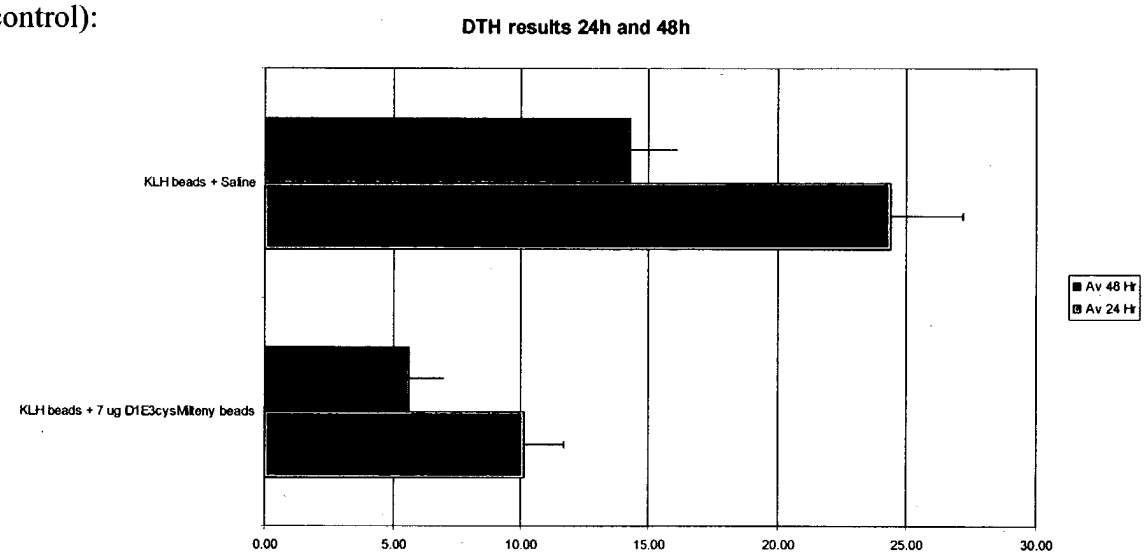
As the data demonstrates, mice receiving the D1E3C/dextran conjugate showed a significantly decreased DTH response at 24 hours compared to control.

(b) Delivery on Microbeads

As an alternative formulation, the Delta1 protein fragment D1E3C was coupled to iron oxide microbeads from Miltenyi Biotec by reductive coupling. The beads used were iron-dextran particles with a mean particle diameter of approximately 50 nm.

6-8 weeks old female Balb/c mice were again injected sub-cutaneously at the base of the tail with 2 x 10^6 KLH coated beads (prepared as described above) per mouse. Particles bearing D1E3C-coupled beads, as above (approximately 7 μg protein per mouse) were injected sub-cutaneously in a close separate site of the tail base (all agents were administered as aqueous solutions;100 mM sodium phosphate at pH 7). Mice were challenged after 7 days in the right ear with 20 μg of KLH. The increase in ear swelling (right ear – left ear) was measured for the following four days using a digital calliper.

Results are shown below (mice which received KLH beads alone are shown as a control):



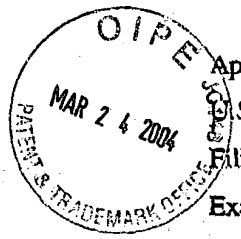
3. These experiments demonstrate that a variety of modes of administration are effective in delivering a Notch ligand and affecting T-cell activation and thus, that the invention is not restricted to any particular method of administration. In particular, it is not necessary for the Notch ligand to be expressed on the surface of an APC or T-cell, as these results clearly demonstrate that Notch ligand conjugated to beads and polymers also produces the desired effect *in vivo*. Therefore, it is respectfully submitted that the data contained herein directly refutes the enablement rejection of claims 48-51 under 35 U.S.C. §112, first paragraph, in the Office Action, and reconsideration and withdrawal of the rejection are solicited.

4. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further, that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: _____

_____
Brian R. Champion

PATENT
674525-2001



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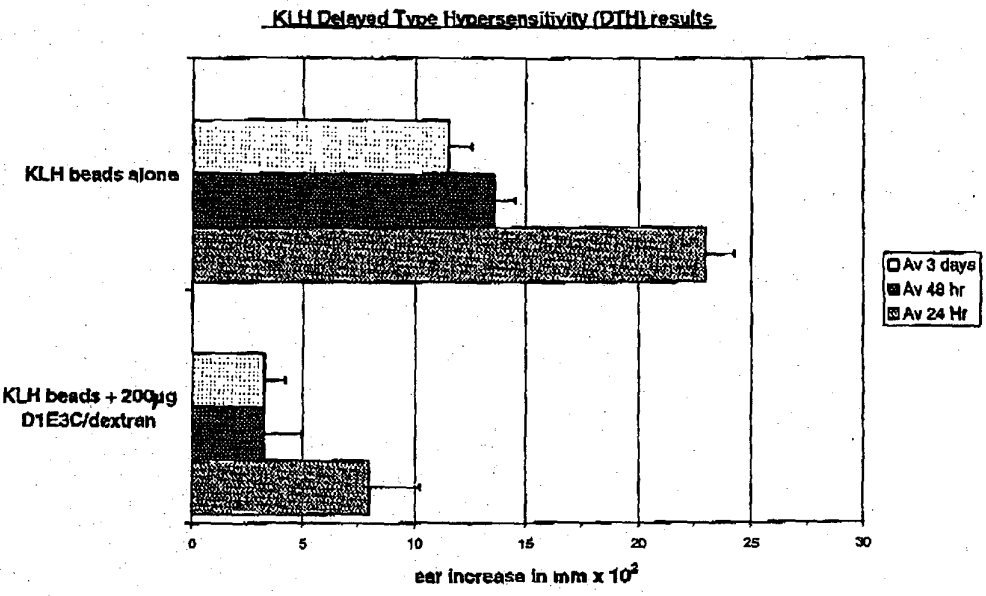
Mice were challenged after 7 days in the right ear with 20 μg of KLH. The increase in ear swelling (right ear – left ear) was measured for the following three days using a digital calliper. Results are shown below (mice which received KLH beads alone are shown as a control):



As the data demonstrates, mice receiving the D1E3C/dextran conjugate showed a significantly decreased DTH response compared to control.

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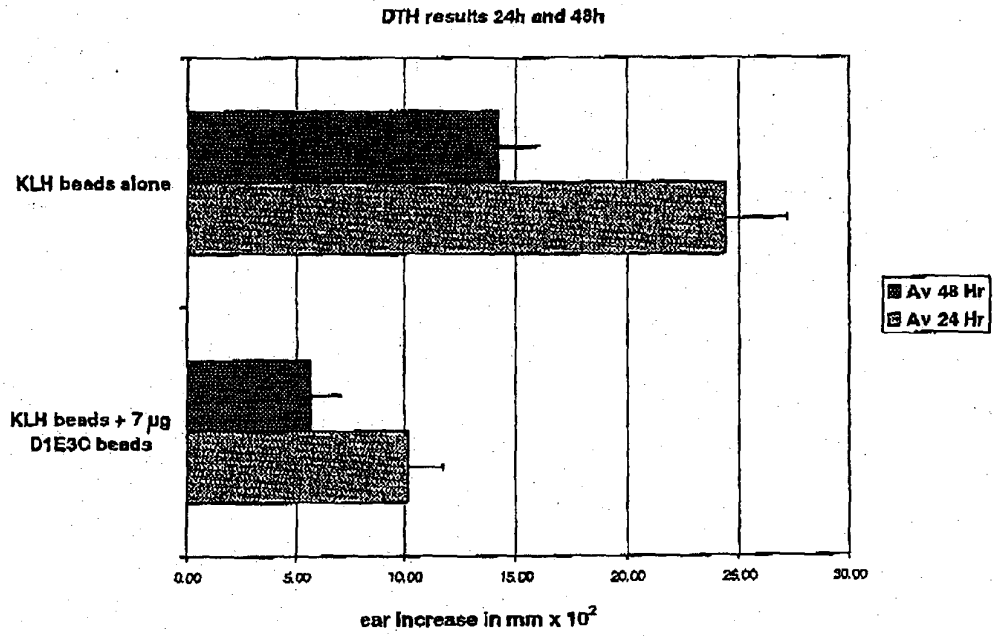
Mice were challenged after 7 days in the right ear with 20 μg of KLH. The increase in ear swelling (right ear – left ear) was measured for the following two days using a digital calliper.

Results are shown below (mice which received KLH beads alone are shown as a control):



This data demonstrates that mice receiving the D1E3C conjugated to microbeads showed a significantly decreased DTH response compared to control.

3. These experiments demonstrate that a variety of modes of administration are effective in delivering a Notch ligand and affecting T-cell activation and thus, that the invention is not restricted to any particular method of administration. In particular, it is not necessary for the Notch ligand to be expressed on the surface of an APC or T-cell, as these results clearly demonstrate that Notch ligand conjugated to beads and polymers also produces the desired effect *in vivo*. Therefore, it is respectfully submitted that the data contained herein directly refutes the enablement rejection of claims 48-51 under 35 U.S.C. §112, first

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Date: 22nd March 2004

Brian R. Champion